Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supporting Information

Peptide-templated gold nanoclusters as a novel label-free biosensor for the detection of protease activity

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EXPERIMENTAL SECTION

Chemicals and Materials.

Porcine pancreatic elastase (from porcine pancreas lyop), MMP-14, trypsin, thrombin, pepsin, elafin, bovine serum albumin (BSA), $HAuCl_4 \cdot 3H_2O$ and sodium borohydride ($NaBH_4$) were purchased from Sigma-Aldrich (Missouri, USA). 3-mercaptopropionic acid (MPA) was purchased from Thermo Fisher Scientific (New Jersey, USA). All other chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Solutions were prepared using ultrapure water, which was obtained from a Millipore Milli-Q water purification system (Billerica, MA, USA) and had an electric resistance >18.3 MΩ. All glassware was washed by aqua regia (conc. HCl: conc. HNO_3, volume ratio = 3:1), and then rinsed with ultrapure water and ethanol. Elastase substrate peptide (CCAAAA) and control peptides (CCGGGG and CCGSGS) were synthesized and purified (>95% purity) by A' Peptide Co. Ltd. (Shanghai, China).

Synthesis of peptide-protected AuNCs

In a typical experiment, a solution of peptide (200 μL, 2 mM) was mixed with a solution of $HAuCl_4$ (3.0 mL, 2% by mass), followed by the addition of a solution of 3-mercaptopropionic acid (300 μL, 30 μM in MeOH) under stirring at room temperature. 10 min later, a solution of $NaBH_4$ (10 M, 300 μL) was added and the mixture was stirred at room temperature for 10 min, during which time the solution changed from gray to colorless, indicating the formation of the peptide-protected Au nanoclusters. The solution was utrafiltrated (MerckMillipore, Amicon Ultra-0.5, MWCO: 3000) to remove the free peptide and 3-mercaptopropionic acid.

Characterization of Au NCs

High resolution transmission electron microscopy (HRTEM) images of peptide-protected AuNCs were obtained using a JEM-2000FX with an operating voltage of 200 kV. The absorption spectrum of CCAAAA-AuNCs was recorded on a UV-Vis absorption spectroscopy (Shimadzu UV-2450, Kyoto, Japan). Fluorescence measurements were carried out using a Fluorolog-Tau-3 spectrofluorometer (Jobin

Yvon Inc., NJ).

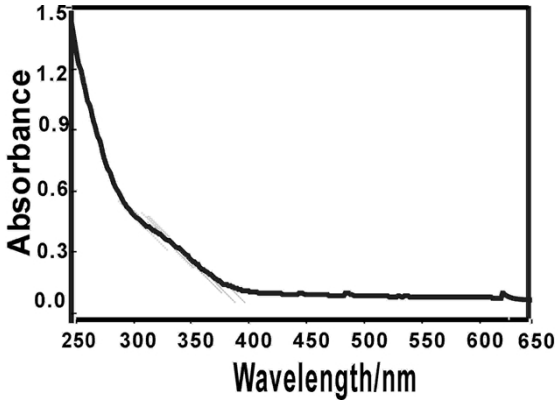

Fig. S1. UV-Vis absorption spectra of peptide1–AuNCs .

Measurement of the quantum yield for CCAAAA-AuNCs.

Quinine bisulfate was used as reference. The integrated fluorescence intensity at the emission peak and the absorbance were used to calculate the quantum yield of CCAAAA-AuNCs from the following eqation:

$$\varphi_X = \varphi_{ST}\left(\frac{I_X}{I_{ST}}\right)\left(\frac{A_X}{A_{ST}}\right)\left(\frac{n_X}{n_{ST}}\right)^2$$

Where φ_x is the quantum yield of the sample, φ_{ST} is the quantum yield of the reference compound, I_X and I_{ST} are the integrated fluorescence intensity at the emission peak of the sample and reference respectively, A_x and A_{ST} are the absorbance of the sample and reference at the specific wavelength used for fluorescence excitation respectively. n_X (H_2O, 1.3329) and n_{ST} (methanol, 1.3611) are the refractive indices of the solvents. According to this equation, the quantum yield of CCAAAA-AuNCs was 15.8%.

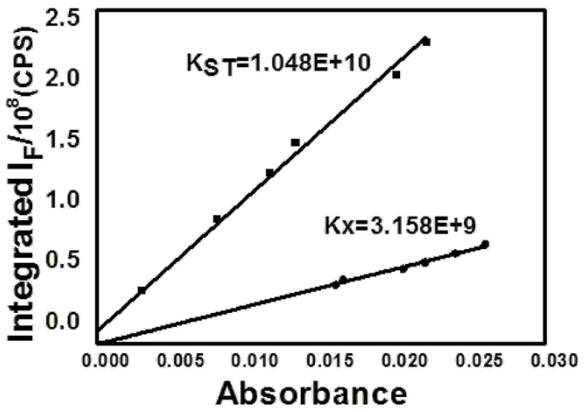

Fig. S2. Plot of the integrated fluorescent intensity versus the absorption for the standard (black) and peptide1-Au NCs (red). The gradient for each sample is proportional to that sample's fluorescence quantum yield.

Characterization of the hydrolysis products of the surface peptide on AuNCs

A solution of CCAAAA-AuNCs (100 μM, 100 μL, before and after treatment with elastase) was mixed with a solution of KCN and $K_3(FeCN)_6$ (2 mM for each, 200 μL) to digest the Au core of the nanoclusters. The mixture was allowed to stand at room temperature for 1h. The mass spectra of the mixture were acquired on a liquid chromatography-electrospray mass spectrometer (Orbitrap Velos Pro, Thermo Fisher Scientific, USA).

Fluorescence anisotropy measurements on AuNCs

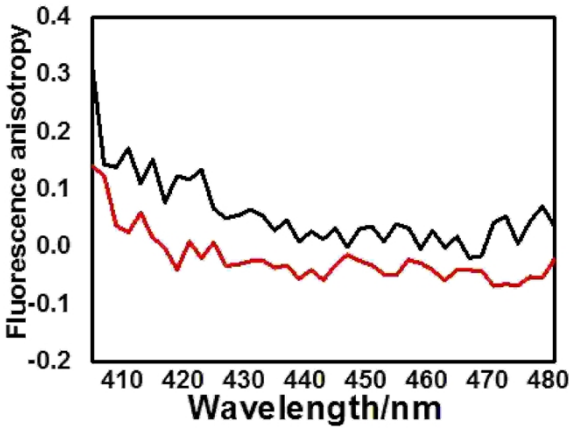

Fig. S3. Fluorescence anisotropy profiles of CCAAAA-AuNCs before (black) and after (red) their incubation with elastase.

Experiment of peptide-AuNCs 's fluorescence quenching mechenism

For the control experiment to explore the fluorescence quenching mechenism of peptide1-AuNCs induced by elastase. We firstly saturated the 100 μL aqueous solution of peptide1-AuNCs with nitrogen (N_2) in place of air for about 15 min. Then 10 μL reaction buffer (PB, pH 7.2, 100 mM) and 1 μL 100 nM of elastase were

introduced into the N_2-saturated solution of peptide 1-AuNCs and incubated at 25° C for 40 min under the anaerobic condition. The resulting solution and air-saturated peptide 1-AuNCs solution incubated with elastase were immediately subjected to fluorescence spectral measurements respectively.

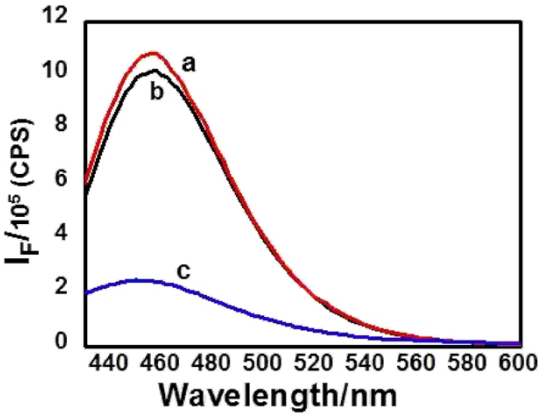

Fig. S4. Fluorescence quenching effect of oxygen on elastase-treated CCAAAA-AuNCs. (a) CCAAAA-AuNCs solution; (b) N2-saturated CCAAAA-AuNCs solution incubated with elastase; (c) Air-saturated CCAAAA-AuNCs solution incubated with elastase.

Fluorescence detection of elastase

10 μL of phosphate buffer (pH 7.2, 100 mM) was added to 100 μL of CAAAA-AuNCs solution , followed by the addition of 1μL of elastase at designated concentration. The mixture was incubated at 25°C for 40 min. The resulting solution was immediately subjected to fluorescence measurements.